265 Impact of two antiretroviral regimens on fecal microbial diversity and composition

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Introduction

- Gut microbial composition is altered in chronic HIV-infected individuals naïve to antiretroviral therapy (ART)[1-4]
- The microbial composition of HIV-infected individuals on ART is different from that of non-HIV-infected individuals [1,4]
- The degree to which ART restores the gut microbial composition remains unclear[4].
- Deciphering the effects of different ARV regimens on gut microbiota composition is of great interest to develop therapies aimed at restoring the latter to a non-HIV state.

Aim:
Determine the effect of two ARV regimens on fecal microbial diversity and composition:

- **EFV-based regimen** (EFV)

Efavirenz/Emtricitabine/Tenofovir disoproxil fumarate

- **Protease inhibitor-based regimen** (PI)

Emtricitabine/Tenofovir DF boosted with either Lopinavir/ritonavir (LPV/r) or Atazanavir/r (ATV/r)

Table 1: Description of cohorts

Group	HIV-	EFV	PI
N	9	20	17
Age (years)	38 (20-66)	42 (24-59)	40 (19-54)
Female (%)	4 (44.4%)	2 (10%)	2 (10%)
CD4 (cells/mm^3)	965 (617-1468)	457 (235-1247)	559 (240-1177)

Values are expressed as median (range). All individuals on ART had undetectable viral load (<40 RNA copies/mL). Median duration of ART was 67 ± 32.5 and 75 ± 35.7 months for EFV- and PI-based regimen respectively (p>0.05).

Methodology

- All individuals gave informed consent. The protocol was approved by our local ethics committee (CCBI-INER).
- Fecal samples were obtained from 9 non-HIV-infected and 37 HIV-infected individuals on ART.
- The V3 region of the 16S rRNA gene was PCR amplified in triplicate using barcoded primers. V3 amplicons were sequenced on the Ion Personal Genome Machine (PGM) using the 200bp chemistry (Life Technologies).
- Raw reads were quality- and size-filtered using an modified version of *cutadapt*[5]. Filtered reads were analyzed using QIIME 1.8.0[6] for taxonomic assignment and diversity analyses.
- Statistical significance was determined using non-parametric Kruskall-Wallis and /or Mann-Whitney U-test (GraphPad Prism 6).

The fecal microbial composition at phylum level is different in individuals on two different ARV regimens

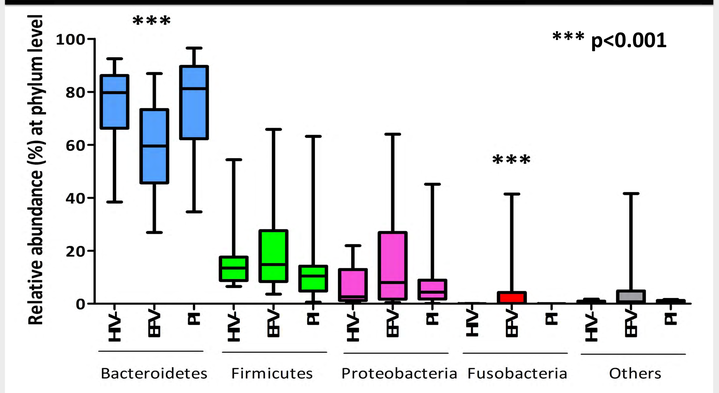


Figure 1: Microbial communities were profiled at each taxonomic level (phylum to genus) using QIIME 1.8.0 pick_open_reference_otus.py script, uclust-based assigner and Greengenes 13.5 reference database. Four predominant phyla were found: Bacteroidetes, Firmicutes, Proteobacteria and Fusobacteria. Overall relative abundance for each phylum was analyzed using Kruskal-Wallis test. Bacteroidetes were significantly reduced in individuals receiving EFV- as compared to those receiving PI-based regimen (KW p=0.0080; KW stats=9.647). Fusobacteria were only observed in individuals under EFV-based regimen (KW p=0.0004; KW stats=15.80).

Individuals on EFV have significantly lower fecal microbial diversity

	Observed Species	PD*	Chao1	Shannon
HIV-	1625 ± 279.5	100 ± 15.8	4420 ± 776.4	5.2 ± 0.42
EFV	1246 ± 425.9	78 ± 20.41	3304 ± 1037.9	4.6 ± 0.76
PI	1502 ± 356.6	93 ± 18.41	4287 ± 1215.7	5.0 ± 0.64
p value (HIV- vs EFV)	0.030	0.0036	0.0036	0.0231
p value (EFV vs PI)	0.0304	0.0184	0.0184	0.1761
p value (HIV- vs PI)	0.5227	0.3114	0.8688	0.2627

Table 2: Four alpha diversity indices were calculated: observed species, *phylogenetic diversity (PD), Chao1 and Shannon`s index. Samples were rarified at 49,208 sequences/sample to avoid bias. Values are given as mean ± standard deviation. p values below 0.05 (Mann-Whitney U test) are shown in bold.

References

1. Lozupone C.A. *et al.* Alterations in the gut microbiota associated with HIV-1. *Cell host Microbe* **14**, 329-39 (2013)
2. Vujkovic-Cvijin I *et al.* Dysbiosis of the gut microbiota is associated with HIV disease progression and tryptophan catabolism. *Sci Transl Med* **5**, 193ra91 (2013)
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4. Lozupone C.A. *et al.* HIV-induced alteration in gut microbiota: driving factors, consequences, and effects of antiretroviral therapy. *Gut Microbes* **5**, 562-70 (2014)
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6. Caporaso et al. QIIME allows analysis of high-throughput community sequencing data. *Nature Methods* **7**, 335-336 (2010)

Effect of two different ARV regimens on the fecal microbiota composition at genus level

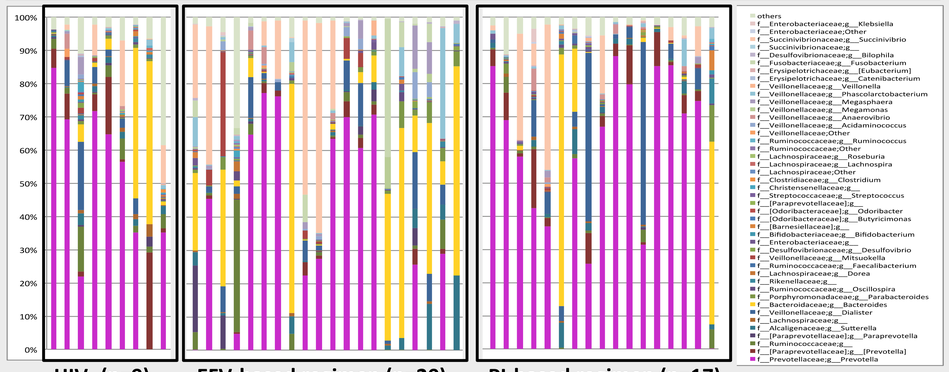


Figure 2: Taxonomy bar charts showing the relative abundance (%) at genus level. The predominant genera are Prevotella (shown in pink) and Bacteroides (shown in yellow).

Taxa that are differentially abundant in the ART-treated cohort: Prevotella and Bacteroides

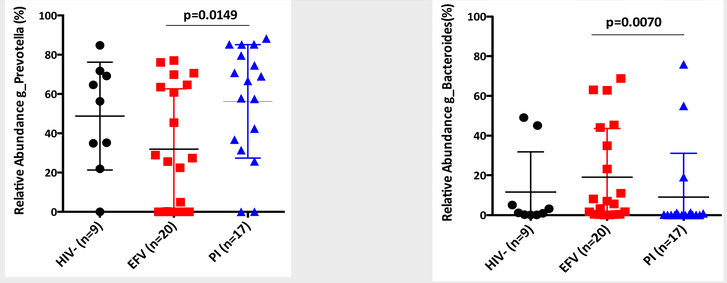


Figure 3: The abundance of Prevotella was significantly decreased while the abundance of Bacteroides was significantly increased in individuals on EFV-based regimen compared to PI-based regimen (belonging to the Bacteroidetes phylum). Mann-Whitney U test.

Conclusion

- ART-treated individuals on EFV-based regimen had significantly lower fecal microbiota diversity as compared to PI-based regimen (Table 2).
- Individuals on a EFV-based regimen have a different fecal microbiota composition that is Prevotella-poor and Bacteroides-rich compared to those individuals on a PI-based regimen (Fig.3).
- Differences in the Prevotella/Bacteroides gradient was not due to differences in diet. Dietary information was collected and most individuals reported a diet high in saturated fat, normal in protein, normal in carbohydrates and low in fiber. Furthermore, no differences in body mass index and calorie intake were observed between groups (data not shown, p>0.05).

Acknowledgments